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Reverse transcription of RNA to cDNA

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Protocol status: Working
We use this protocol and it's working

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Abstract

Synthesizing cDNA (for qPCR)

Guidelines

Always work with gloves and safety gear and work on ice

Materials

- 100 ng RNA
- 40x yellow sample buffer (from DyNAmo Color Flash Kit)
- random hexamer primers
- 5x RT-buffer
- dNTP Mix (10 mM of each nucleotide)
- RevertAid Reverse Transcriptase
- **You will require 100 ng for RT and 100 ng for -RT control (include a -RT control for each sample!)**

Preparation of 1.33X yellow buffer

1 33,25 µL 40x yellow sample buffer + 966,75 µL H2O

optional: This was used to be able to see the pipetting scheme for qPCR more easily

Start

2 In PCR stripes, pipet in the following order:

100 ng template RNA
1 µL random hexamer primers
RNase-free H2O to 13 µL volume

3 Prepare a Mastermix of the following reagents:

4 µL 5x RT-buffer
2 µL dNTP Mix (10 mM of each nucleotide)
1 µL RevertAid Reverse Transcriptase

4 Add 7 µL Mastermix to each reaction

5 For your -RT control, pipet 100 ng RNA in PCR stripes, leave out buffer and everything, just add H2O to a final volume of 20 µL. Mark as -RT so you can distinguish it from your actual cDNA!

PCR

6

PCR protocol:

	10 min	25 °C
	60 min	42 °C
	10 min	70 °C

7 Add 60 µL 1,33 x yellow sample buffer to each reaction

optional: This was used to be able to see the pipetting scheme for qPCR more easily

8 cDNA can be stored at 4 ˚C for a short time, otherwise, freeze at -20 ˚C